

CLAIMS

1. A method for quantitating squalene formed by squalene synthase, comprising:

- a) contacting NADPH, FPP and a magnesium ion cofactor with a squalene synthase;
- b) exposing the reaction mixture to UV light; and
- c) detecting the emitted fluorescent light,

wherein the decrease in the amount of fluorescent light is correlated to the amount of NADPH consumed in the synthesis of squalene, and

wherein the amount of squalene is correlated to the amount of NADPH consumed.

2. A method for determining squalene synthase activity, comprising:

- a) contacting NADPH, FPP and a magnesium ion cofactor with a squalene synthase;
- b) exposing the reaction mixture to UV light; and
- c) detecting the emitted fluorescent light,

wherein the decrease in the amount of fluorescent light is correlated to the amount of NADPH consumed in the synthesis of squalene, and

wherein the amount of squalene is correlated to the amount of NADPH consumed.

3. The method of claim 2, wherein the activity of a squalene synthase is compared to a control.

4. The method of claim 2, wherein the squalene synthase is a plant squalene synthase.

5. The method of claim 4, wherein the plant squalene synthase is an *Arabidopsis* squalene synthase.

6. The method of claim 5, wherein *Arabidopsis* squalene synthase has the amino acid sequence of SEQ ID NO: 6.

7. The method of claim 5, wherein *Arabidopsis* squalene synthase is at least 80% identical to the amino acid sequence of SEQ ID NO: 6.

8. The method of claim 7, wherein *Arabidopsis* squalene synthase is at least 85% identical to the amino acid sequence of SEQ ID NO: 6.

9. The method of claim 8, wherein *Arabidopsis* squalene synthase is at least 90% identical to the amino acid sequence of SEQ ID NO: 6.

10. The method of claim 9, wherein *Arabidopsis* squalene synthase is at least 95% identical to the amino acid sequence of SEQ ID NO: 6.

11. The method of claim 2, wherein the wavelength of the UV light is approximately 330-350 nm and the wavelength of the fluorescent light emission is approximately 465 nm.

12. The method of claim 2, wherein the NADPH is present in the reaction mixture at an initial concentration of 0.0005 mM to 0.5 mM.

13. The method of claim 2, wherein the magnesium ion cofactor is present in said reaction mixture at an initial concentration of 0.5 mM to 100 mM.

14. The method of claim 2, wherein the FPP is present in the reaction mixture at an initial concentration of 0.001 mM to 1 mM.

15. The method of claim 2, wherein said reaction mixture further comprises 75-150 mM phosphate buffer at a pH of 7.0-8.0.

16. A method for identifying a test compound as an inhibitor or promoter of squalene synthase, comprising:

a) contacting NADPH, FPP and a magnesium ion cofactor with a squalene synthase in the presence and in the absence of a test compound;

b) exposing the reaction mixture to UV light; and

c) detecting the emitted fluorescent light over time,

wherein the decrease in the amount of fluorescent light over time is correlated to the amount of NADPH consumed in the synthesis of squalene, and

wherein the amount of squalene produced over time is correlated to the amount of NADPH consumed, and

wherein an increase in the amount of fluorescent light emission over time in the presence of the test compound indicates that the test compound is a squalene synthase inhibitor, and

wherein a decrease in the amount of fluorescent light emission over time in the presence of the test compound indicates that the test compound is a squalene synthase promoter.

17. The method of claim 16, wherein the squalene synthase is a human squalene synthase.

18. The method of claim 16, wherein the squalene synthase is a fungal squalene synthase.

19. The method of claim 16, wherein the squalene synthase is a plant squalene synthase.

20. The method of claim 19, wherein the plant squalene synthase is an *Arabidopsis* squalene synthase.

21. The method of claim 20, wherein the *Arabidopsis* squalene synthase has the amino acid sequence of SEQ ID NO: 6.

22. The method of claim 20, wherein the *Arabidopsis* squalene synthase is at least 90% identical to the amino acid sequence of SEQ ID NO: 6.

23. The method of claim 20, wherein the *Arabidopsis* squalene synthase is at least 95% identical to the amino acid sequence of SEQ ID NO: 6.

24. The method of claim 16, wherein the wavelength of the UV light is approximately 330-350 nm and the wavelength of the fluorescent light emission is approximately 465 nm.

25. The method of claim 16, wherein the NADPH is present in the reaction mixture at an initial concentration of 0.0005 mM to 0.5 mM.

26. The method of claim 16, wherein the magnesium ion cofactor is present in the reaction mixture at an initial concentration of 0.5 to 100 mM.

27. The method of claim 16, wherein the FPP is present in the reaction mixture at an initial concentration of 0.001 mM to 1 mM.

28. The method of claim 16, wherein the reaction mixture further comprises 10-100 mM Tris-HCl buffer at a pH of 7.0-8.0.

29. A method for identifying compounds capable of selectively promoting or inhibiting plant, fungal and/or animal squalene synthase activity, comprising:

a) combining FPP, NADPH, a magnesium ion cofactor and a plant squalene synthase to form a reaction mixture under conditions suitable for the production of squalene in the presence and absence of a test compound;

b) subjecting the reaction mixture to UV light and detecting fluorescent light emission over time,

c) determining the activity of the compound to promote or inhibit squalene synthase based on the fluorescent light emission over time,

d) repeating steps a-c using a fungal or animal squalene synthase, and

f) identifying compounds that selectively inhibit plant, fungal or animal squalene synthase.

30. The method of claim 29, wherein the squalene synthase is a plant squalene synthase.

31. The method of claim 30, wherein the plant squalene synthase is an *Arabidopsis* squalene synthase.

32. The method of claim 31, wherein the *Arabidopsis* squalene synthase has the amino acid sequence of SEQ ID NO: 6.

33. The method of claim 31, wherein the *Arabidopsis* squalene synthase is at least 90% identical to the amino acid sequence of SEQ ID NO: 6.

34. The method of claim 31, wherein the *Arabidopsis* squalene synthase is at least 95% identical to the amino acid sequence of SEQ ID NO: 6.

35. The method of claim 29, wherein the wavelength of the UV light is approximately 330-350 nm and the wavelength of the fluorescent light emission is approximately 465 nm.

36. The method of claim 29, wherein the FPP is present in the reaction mixture at an initial concentration of 0.0001 mM to 1 mM.

37. The method of claim 29, wherein the NADPH is present in the reaction mixture at an initial concentration of 0.0005 mM to 0.5 mM.

38. The method of claim 29, wherein the magnesium ion cofactor is present in the reaction mixture at an initial concentration of 0.5 mM to 100 mM.

39. The method of claim 29, wherein the reaction mixture further comprises 10-100 mM Tris-HCl buffer at a pH of 7.0-8.0.

40. The truncated squalene synthase having the sequence of SEQ ID NO: 6 or SEQ ID NO: 6 with conservative substitutions.

41. A polypeptide having squalene synthase activity, wherein said polypeptide is at least 90% identical to the amino acid sequence of SEQ ID NO: 6.

42. The polypeptide of claim 41, wherein said polypeptide is at least 95% identical to the amino acid sequence of SEQ ID NO: 6.

43. The oligonucleotide sequence of SEQ ID NO: 5 or a degenerative variant thereof.

44. An isolated nucleic acid comprising a sequence that encodes squalene synthase comprising an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 6.

45. The nucleic acid of claim 44, wherein the amino acid sequence has at least 95% identity with SEQ ID NO: 6.

46. The nucleic acid of claim 44, wherein the squalene synthase has at least 50% of the activity of the squalene synthase identified by SEQ ID NO: 6.

47. The nucleic acid of claim 44, wherein the squalene synthase has at least 60% of the activity of the squalene synthase identified by SEQ ID NO: 6.

48. The nucleic acid of claim 44, wherein the squalene synthase has at least 80% of the activity of the squalene synthase identified by SEQ ID NO: 6.

49. The nucleic acid of claim 44, wherein the squalene synthase has at least 90% of the activity of the squalene synthase identified by SEQ ID NO: 6.